

What is claimed is:

1. A process for the quantitative determination of 25-hydroxy-cholecalciferol in animal feed which comprises the steps of
  - a) dispersing the feed sample in water and adding to the sample a defined amount of an internal standard compound having a mass different from 25-hydroxycholecalciferol and having a polarity similar to but different from 25-hydroxycholecalciferol;
  - b) extracting the aqueous dispersion with tert.butyl methyl ether;
  - c) submitting the ether extract to semipreparative HPLC;
  - d) collecting the fractions containing 25-hydroxycholecalciferol and the internal standard compound;
  - e) submitting the fractions collected in d) or an aliquot thereof to HPLC combined with mass spectrometry;
  - f) determining the MS peak areas of 25-hydroxycholecalciferol and of the internal standard compound added; and
  - g) calculating the amount of 25-hydroxycholecalciferol by computing the MS peak areas measured.
2. A process as in claim 1 wherein the standard compound is 26,27-hexadeutero-25-hydroxycholecalciferol, 25-hydroxy-ergocalciferol, or 1 $\alpha$ -hydroxy-cholecalciferol.
3. A process as in claim 2 wherein the standard compound is 26,27-hexadeutero-25-hydroxycholecalciferol.
4. A process as in any one of claims 1-3 wherein the semipreparative HPLC is carried out on silica gel as the stationary phase and an isopropanol:ethyl acetate:isooctan mixture as the mobile phase.

5. A process as in claim 4 wherein the mobile phase is isopropanol:ethyl acetate:isooctan in a ratio (by volume) of about 1 : 10 : 89.
- 5 6. A process as in claim 4 or 5 wherein the stationary phase is Hypersil Si 60, 3  $\mu$ m.
7. A process as in any one of claims 1-6 wherein the analytical HPLC is carried out in a chromatography system comprising a trapping column on which the substances to be measured are concentrated, and the intrinsic analytical column for separation.
- 10 8. A process as in claim 4 wherein the stationary phase in the analytical HPLC is a modified silica gel such as Aquasil C18, 3  $\mu$ m.
- 15 9. A process as in claim 7 or 8 wherein a gradient of water containing 0.05 % (vol/vol) formic acid and methanol containing 0.05 % (vol/vol) formic acid is used as the mobile phase.

\*\*\*